BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/76662 A2

(51) International Patent Classification[7]: B01L

(21) International Application Number: PCT/US00/16056

(22) International Filing Date: 12 June 2000 (12.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/138,964 11 June 1999 (11.06.1999) US
09/592,365 12 June 2000 (12.06.2000) US

(71) Applicant: ORCHID BIOSCIENCES, INC. [US/US]; 303 College Road East, Princeton, NJ 08540 (US).

(72) Inventors: SWENSON, Rolf, E.; 35 Fieldston Road, Princeton, NJ 08540 (US). JIAN, Lin; 6 Lois Drive, Walpole, MA 02081 (US). DEWITT, Sheila, H.; 346 Bunker Hill Road, Auburn, NH 03032 (US).

(74) Agent: MIERZWA, Kevin, G.; Artz & Artz P.C., Suite 250, 28333 Telegraph Road, Southfield, MI 48034 (US).

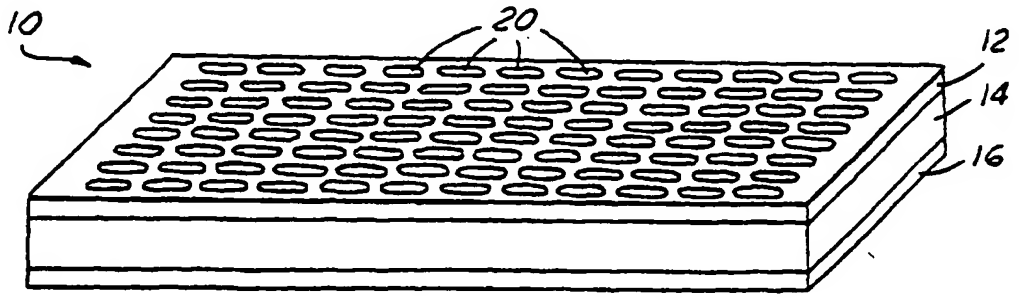
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROENABLED CHEMICAL REACTION IN MICROFLUIDIC CHIPS



(57) Abstract: A chemical process to obtain products of higher purity or in shorter time is provided by performing chemical reactions in a reactions volume less than 1 microliter. The advantages of this process are due to better mixing or heat exchanche (each 10 fold decrease in reaction volume leads to a 100-fold improvement mixing or heat exchange) or better surface to volume ratio. Better surface to volume ratio is a major factor in reactions critically dependent on surface mediated events such as heterogeneous catalysis. This increase in yield and speed of reaction can be conducted in parallel sets of reaction chambers provided by multilayer glass and silicon chips. Reagents can be added via a microfluidic chip incorporating channels and orthogonal row/column reagent delivery options. This discovery is amendable to solid supported synthesis as well as solution synthesis. Because of the better surface to volume ratio, this process is particularly useful with solid catalyst on the edges of the reaction chamber and can be used for hydrogenation, and hydroformulation. This process is demonstrated in reactions involving nucleophilic aromatic substitution on a solid support.

WO 00/76662 A2

MICROENABLED CHEMICAL REACTION IN MICROFLUIDIC CHIPS

Cross-Reference to Related Applications

The present invention is related to copending provisional application Serial No. 06/138,964, entitled, "Micro-enabled Syntheses in Micro-fluidic Chips", filed on June 11, 2000, which is hereby incorporated by reference.

Technical Field

The present invention relates to fluid sample processors, particularly those used in combinatorial chemistry and DNA synthesis.

Background Of The Invention

There are several multiple fluid sample processors known today, particularly those which are micro in size and are able to carry out from dozens to hundreds of experiments and analyses simultaneously. These devices, often called microfluidic devices, have particular use in combinatorial chemistry and DNA synthesis. These devices provide discovery and diagnostic tools which increase the speed and productivity of discovering new drug candidates and analyzing DNA materials, and do so on a miniaturized scale or platform that reduces cost and manual handling.

Many of the known devices utilize a plurality of layers, such as a feed-through layer, a fluidic delivery layer, and a well plate layer. A network of apertures and passageways in the various layers allow passage and transport of various materials and reagents to specific channels and wells for processing. Various mechanisms, such as electro-

osmosis or pressure pumping precisely control the flow of materials in the processor.

These devices typically have a network or grid of openings and wells, arranged in rows and columns. Typically, materials added to the processor such as reagents are utilized to fill or couple with an entire row or an entire column of wells and reservoirs.

A heightened interest in microreactors and associated operations recently has been in response to pressures from preclinical drug discovery and chemical development/manufacturing. The economic pressures on the pharmaceutical industry to provide higher quality therapeutics in a shorter amount of time at reduced cost has driven adoption of several new technologies and components thereof, including, combinatorial chemistry and high throughput screening. Also, efforts to increase efficiency and productivity of chemical synthesis efforts have leveraged parallel reactor systems and automation for both medicinal chemistry and process optimization. Miniaturization of chemical reactors have several benefits including decreased reagent and processing costs, improved process conditions such as heat transfer, improved conversion and selectivity, penetration of thermal runaway reactions, control of free radical branching reactions, increased safety as a result of the use of smaller volumes and enhanced temperature control, lower waste volume streams, control of atmospheric conditions that limit reagent degradation and evaporation and the ability to provide multiple small reactors versus a single large

reactor. In most situations, the manufacturing efforts have focused on serial processing microreactors. Because these efforts have been directed toward high throughput screening efforts in support of lead generation efforts, they have focused on the production of a variety of products simultaneously. Commonly, open well formats have been used. Disadvantages to the open well format include that they are limited to chemistries that are insensitive to air, moisture, evaporation, and mixing. Therefore, these systems are typically limited to oligomeric syntheses including peptides or oligonucleotides.

While particular embodiments of the invention have been shown and described, numerous variations alternate embodiments will occur to those skilled in the art. Accordingly, it is intended that the invention be limited only in terms of the appended claims.

Summary Of The Invention

It is an object of the present invention to provide a new and improved multiple fluid sample processor, system and method, particularly for use in various types of chemical synthesis including oligomeric synthesis including DNA, peptides, oligosaccharides and other repetitive chemical or biological processes. It is another object of the present invention to provide a system and method for forming chemical products in parallel in a column and row format in a multiple fluid sample processor.

It is another object of the invention to provide a liquid handling diagnostic and analysis tool which increases the speed and productivity of synthesis, discovery of new drug candidates, primers or probes for genotyping, and antigen or epitope identification, and to do so on a miniaturized scale or platform that reduces cost and manual handling.

Other objects, purposes, and advantages of the present invention will become apparent in the following description of the invention, particularly when viewed in accordance with the attached drawings and appended claims.

In accordance with the present invention, a multiple fluid sample processor, system, and method are provided which utilizes a multi-layered fluidic array having micro-sized reservoirs, connecting micro channels and reaction cells and wells. Micro-sized wells typically range in sizes from 10 nl to 10 μl and more particularly from 100 nl to 1 μl. Micro-sized channels typically range in diameter from 10 microns to 5 millimeters and more particularly from 50 microns to 1 millimeter. A three-dimensional architecture of micro channels and micro-reaction vessels are constructed in the layers in order to transport reagents and other materials throughout the structure.

For a multi-layered device, the array preferably includes a top feed-through plate, a middle distribution plate, and a bottom well plate. The top feed-through plate serves as a cover for the array and contains micro-channels which direct

materials to apertures selectively positioned above reservoirs located in the central distribution plate or layer. The apertures are in communication with micron-size reservoirs, micro channels, reservoir feeds, cell feeds, and overflow feeds, which are selectively formed in the center distribution plate. The channels and reservoirs form a delivery system where reservoirs are grouped into elongated columns and rows. In this manner, when a solution or materials is added to one of the apertures in the top plate, it is routed and distributed to fill all of the reservoirs or wells along a column or row in the distribution plate, which could be 6, 8, 10 or more reservoirs or wells. Then the materials in each reservoir or well in that column or row are all treated in the same manner and exposed to the same processing collectively.

Various fluid delivery mechanisms can be utilized to distribute the reactions and other fluids in the display array and to fill the appropriate reservoirs. These mechanisms include pressurized fluid delivery systems, electro-osmosis and electrohydrodynamic distribution.

The present invention provides a system that is used to synthesize various chemical products in parallel.

One advantage of the invention is that yield and purity may be increased of the products produced are increased. This may at least in part due to better heat transfer, mixing and more reactant surface to volume contact.

Another advantage of the invention is that multiple parallel microreactors may be used in place of large chemical facilities.

Brief Description of the Drawings

FIGURE 1 illustrates a multiple fluid sample processor which can be used with the present invention;

FIGURE 2 is an exploded view of the sample processor shown in Figure 1;

FIGURE 3 is a cross-sectional view of the top layer of the processor shown in Figures 1 and 2, the cross-section being taken along line 3-3 in Figure 2;

FIGURE 4 is a cross-sectional view of the middle layer of the processor shown in Figures 1 and 2, the cross-section being taken along line 4-4 in Figure 2;

FIGURE 5 is a cross-sectional view of the bottom or well plate layer of the processor shown in Figures 1 and 2, the cross-section being taken along line 5-5 in Figure 2;

FIGURE 6 is a schematic diagram of the processor showing columns and rows thereof;

FIGURE 7 is a top view of the processor network showing the columns and row.

FIGURE 8 is a plot of the amount of conversion of various amines.

FIGURE 9 is a plot of conversion versus time of various nucleophilic aromatic substitutions for various amines.

FIGURE 10 is a plot of conversion versus time of various nucleophilic aromatic substitutions for amine #48.

FIGURE 11 is a plot of conversion versus time of various nucleophilic aromatic substitutions for amine #49.

FIGURE 12 is a plot of conversion versus time of various nucleophilic aromatic substitutions for amine #21.

Best Mode(s) For Carrying Out The Invention

As indicated above, the present can be used in any synthesis or analysis in which a chemical event takes place. These include various types of chemical synthesis including synthesis of oligonucleotide (DNA) arrays, oligosaccharide arrays, peptide arrays, hydrogenations, hydroformylations or reactions a required gas liquid or solid required, as well as biological arrays.

In peptide synthesis, polypeptides may be synthesized by techniques known to those skilled in the art as, for example, by so-called "solid phase" peptide synthesis or by usual methods of solution phase chemistry. A summary of available solid phase peptide synthetic techniques may be found in Stewart et al., *Solid Phase Peptide Synthesis (W. H.* Freeman Co., San Francisco, *1963)* and Meienhofer, *Hormonal*

Proteins and Peptides, Vol. 2., p. 46 (Academic Press-New York, *1973)*. For classical solution synthesis see Schroder et al., *The Peptides, vol. 1,* (Academic Press - New York, *1965)*.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain bound to a suitable resin. The starting amino acids are commercially available or can be synthesized in any conventional manner, where novel in the compounds of this invention, are synthesized by methods detailed below from readily available or can be synthesized in any conventional manner.

A representative multiple fluid sample processor for use in the present invention is shown in Figures 1 and 2, with cross-sections of the layers being shown in Figures 3, 4, and 5. The processor, which is generally referred to by the reference number 10, is a three layer structure in the embodiment illustrated. It is also understood that the processor can include a larger or smaller number of layers, as needed or desired for the particular chemical or DNA operations desired to be performed.

Processor 10 includes a top layer 12, which is also called a reagent reservoir. The processor also includes a middle layer 14, also called a fluidic delivery or distribution layer. The bottom layer 16 is also called a well chip, and includes a plurality of individual wells or containers.

The top layer feeds compounds and materials into the processor 10 and also serves as a cover for it. The layer 12 contains a number of apertures 20, which are selectively positioned immediately above openings 22, 24 in the reservoir or fluidic delivery layer 14. The openings 22, 24 are connected by an elongated micro-channel 26 which, in turn, has a plurality of small passage channels 28.

The bottom or lower plate member 16 has a plurality of reservoirs or wells 30 which are used to hold the reagents and other materials in order for them to chemically react. Each of the reaction wells 30 has an entrance channel 32 and an exhaust or drain channel 34.

The three layers 12, 14, and 16, are stacked together to form a modular configuration. They also are typically coupled together tightly to form a liquid-tight seal. Sealing gaskets or members 15 can be utilized, if necessary. If desired, the top layer 12 can be bounded or fused to the central distribution plate 14. The bottom or well plate is typically detachably coupled to layer 14 or a combination of layers so they can be removed for further processing and/or testing of the materials in the wells 30.

The wells 30 may be coated with a catalytic material depending on the reaction to be performed or the products to be formed. For example, palladium, platinum, nickel or copper nickel may be used.

The plates 12, 14, and 16 can be made from any desirable material, such as glass, fused silica, quartz, or silicon wafer material. The reservoirs, micro-channels and reaction cells are controllably etched or otherwise formed into the plates using traditional semiconductor fabrication techniques with a suitable chemical or laser etchant.

In order to provide a small, cost efficient analytical device, the channels, wells and reaction cells are preferably provided on a micro-sized level. In this regard, the micro-sized wells typically range in size from 10 nl to 10 μl; and more particularly from 100 nl to 1 μl. The cross-sectional dimensions of the micro-channels typically range in size from 10 microns to 5 millimeters, and more particular from 50 microns to 1 millimeter.

A pressure pumping mechanism (not shown) can be used to assist in loading and distributing the reagents and other materials within the layers. After the reagents or other materials are passed through apertures 20 in the top layer 12, the pressure mechanism applies air pressure sufficiently in order to distribute the materials evenly along channel 26 and into each of the reaction reservoirs or wells 30. The pressure exerted by the pressure mechanism conveys the liquids through the small passageways 28 and 32 until the materials reside in the larger reaction wells.

Subsequently, when it is desired to empty or exhaust the materials from the reaction wells, pressure is increased in the pressure mechanism

sufficiently to exhaust materials from the reaction wells. For this purpose, a collection or drain plate (not shown) can be positioned immediately below the processor 10 during its use.

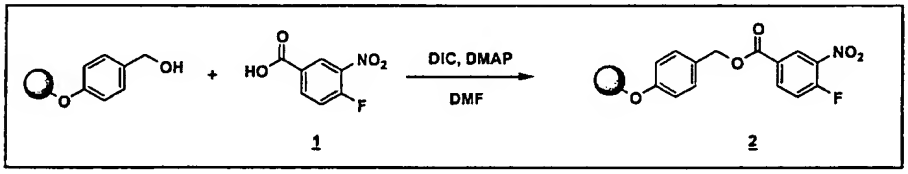
The particular well chip 16 shown in Figures 1 and 2 is a 384-well sample plate. Standard well plates are typically provided in multiples of 96, with a 96-well sample plate being commonly used. Larger multiples of 96 can also be utilized. The detachable layers are preferably of a common dimensionality for ease of handling by robotic or other automation means. A common set of dimensions has been adopted by many manufacturers which match that of a 96-well plate known as a "microtiter" plate. Due to the column and row format of the processor 10, a material entering apertures 22 or 24 and being transferred along channel 26 is introduced into every well 30 along that column or row.

A simple example of the present invention is provided below. Although the sample illustrates the use of the invention with a particular number of columns and rows, it is to be understood that the same rationale and principles can be applied to processors having greater numbers of rows and columns. Also, although the sample illustrates the use of the invention for DNA (oligonucleotide) synthesis, it is to be understood that the invention can be used for numerous other chemical and biological events, such as peptide synthesis, oligosaccharide synthesis, and biological assays.

Figures 6 and 7 show schematically a representative matrix in a processor showing the columns and rows. As shown, each column C and row R has an entrance into a single well W1. The intersections of each of the rows and columns represents a single well. Thus, each well can be served either by a column or row operation.

Generally, the microreactor device is used to synthesize a number of chemical synthesis. At least two reagents, coupling agents or the like are introduced in rows and columns of the microreactor. The essentially same compounds are synthesized in each of the wells. Preferably, the flow of reagents to the wells is stopped during or prior to forming the chemical compound.

Scheme-1

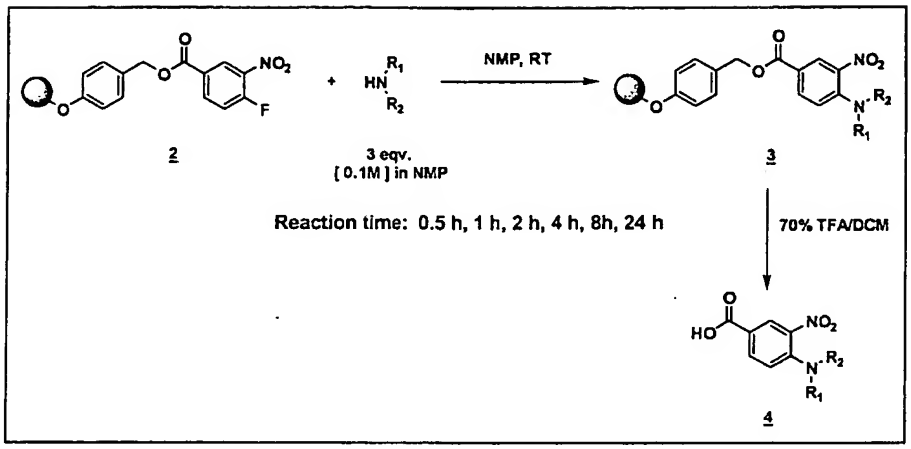


A few examples of synthesis are now illustrated. Wang resins (200-250 μm, 1% divinylbenzene (DVB) crosslinking, 1.7 mmol/g) were obtained from Polymer Laboratories Inc., MA. Other chemicals and anhydrous solvents were obtained from Aldrich, Fluka, Lancaster and Fisher. All reagents and solvents were used without further purification. All synthetic compounds and intermediates gave satisfactory MS. Mass spectrometry and HPLC analyses were performed on PE SCIEX API 2000 LC/MS/MS system.

The kinetic studies of microscale nucleophilic aromatic substitutions were performed on Mark-20 v1.0. Microfluidic processor of the present assignee using Chemtel™ 144 chip of the present assignee.

Synthesis of the solid support-bound halide core is shown in (Scheme-1). To a suspension of Wang resin (1.0g, 1.7mmol, 1.7mmol/g) in 10mL of anhydrous DMF were added 4-fluoro-3-nitrobenzoic acid (0.94g, 5.1mmol), diisopropylcarbodiimide DIC (0.64g, 5.1mmol) and 4-dimethylaminopyridine DMAP (21mg, 0.17mmol). The mixture was shaken at room temperature for 1 h. The reagents and solvent were then drained. The resin was washed with DMF (5mLxl), and then retreated with 4-fluoro-3-nitrobenzoic acid (3 eqv.), DIC (3 eqv.) and DMAP(0.1 eqv.) at room temperature overnight. The resulting resin 2 was filtered and rinsed with DMF (x2), DMF/H20 (1:1, x2), THF (x2), DCM (x2), and dried under reduced pressure. Treatment of resin 2 (20mg) with 50% TFA/DCM for 3 h gave the desired cleavage product, 4-fluoro-3-nitrobenzoic acid: 6.3mg (combined with 2nd cleavage product), 100% recovery yield; >90% HPLC purity; LC-MS m/z = 184 (M-H)-.

Scheme-2



In a second example, macroscale screening of secondary amines is described. That is, SPOS nucleophilic aromatic substitution shown in Scheme-2 below. Fifty secondary amine solutions (0.1M, 0.051mmol) in 1-methyl-2-pyrrolidinone (0.51mL) were prepared. Each of the amine solutions was added to a 1.5 mL-glass vial containing resin 2 (10mg, 0.017mmol). The reactions were allowed to shake at room temperature for 2 h. The reagents were removed with syringes. The resins were rinsed with DCM, MeOH and DCM. The resins were then cleaved with 70% TFA/DCM (100 μL) at room temperature for 3 h. Each of fifty cleavage products was transferred to a sample vial. After evaporation of all reagents and solvents in SpeedVac, the products were collected and analyzed by HPLC and LC-MS. The results were summarized in Table 1 and are shown in Fig. 8.

Table 1. HPLC and LC-MS results of nucleophilic aromatic substitution of 50 secondary amines

Compd#	2° Amines	MS results		HPLC results		
		Prod (MS)	Found (M-H)	SM	Prod. Purity	Unknown
1	N-Methylpropargylamine	234.22	233.2	19%	81	
2	Diethylamine	238.25	237.3		93%	0
3	N-methylpropylamine	238.25	237.3	63%	37%	
4	N-Isopropylmethylamine	238.25	237.3	8%	92%	
5	N-methyl-beta-alaninenitrile	249.23	248.2	2%	98°/a	
6	Piperidine	250.26	249.3		100%	
7	Morpholine	252.23	251.2	8%	92%	
8	4-Methylpiperidine	264.29	263.3	4%	96%	
9	1-Methylpiperazine	265.28	264.3	6%	94%	
10	3-hydroxypiperidine	266.26	265.3	7%	93%	
11	4,4-dimethyloxazolidine	266.26	265.3	100%	0%	
12	N-Methylaniline	272.27	271.3	100%	0%	
13	2-Ethylpiperidine	278.31	277.3	51 %	49%	
14	Heptamethyleneimine	278.31	277.3	5%	95%	
15	1-Ethylpiperazine	279.3	278.3	9%	91%	
16	2-Piperdinemethanol	280.29	279.3	49%	51	
17	2-Methylaminoethyl-1,3-dioxane	282.26	281.3	11%	89%	
18	Indoline	284.26	283.3	98%	2%	
19	N-Allylcyclopentylamine	290.33	289.3	79%	21	
20	1-methyl-4-(methylamino)piperdine	293.33	292.3		100%	
21	bis (2-methoxyethyl) amine	298.3	297.3	49%	51	
22	1,2,3,4-tetrahydroisoquinoline	298.3	297.3		100%	
23	N-ethylbenzylamine	300.32	299.3		76%	24%
24	2-(2-methylaminoethyl)pyridine	301.31	300.3		100%	
25	N-methyl-p-anisidine	302.29	301.3		0%	
26	N-isopropylcyclohexylamine	306.37	305.4		0%	
27	4-chloro-N-Methylaniline	306.71	305.7	100%	0%	
28	1,4-dioxa-8-azaspirodecane	308.3	307.3	3%	97%	
29	(s)-(+)-1-1(2-pyrrolidinylmethyl)pyrrolidinone	319.37	318.4	0%	100%	
30	Ethyl Isonipecotate	322.32	321.3	0%	100%	
31	ethyl-N-piperazineocarboxylate	323.31	322.3	12%	88%	
32	3-(3-pyridylmethylamino)propionitrile	326.32	325.3	56%	44%	
33	1-(2-pyridyl)piperazine	328.33	327.3		72%	28%
34	N-butylbenzylamine	328.33	327.3	100%	0%	
35	5-Nitroindoline	329.27	328.3		0%	
36	1-phenylpiperazine	329.35	328.4		0%	
37	N-ethyl-3,4-(methylenedioxy)aniline	330.3	329.3	100%	0%	
38	N-cyclohexylaniline	340.38	339.4	100%	0%	
39	4-benzylpiperidine	340.39	339.4	3%	97%	
40	1-(2-fluoropheny)piperazine	345.34	344.3	8%	92%	
41	tert-butyl-1-piperazinecarboxylate	351.37	350.4	8%	92%	
42	1-2-methoxyphenyl piperazine	357.37	356.4	7%	93%	
43	N-Benzylglycine ethyl ester	358.36	357.4	88%	12%	
44	Methyl-4-oxo-3-piperdinecarboxylate	358.74	357.7	98%	2%	
45	N-methylhomoveratrylamine	360.37	359.4	8%	92%	
46	N-(diphenylmethyl)methylamine	362.39	361.4	100%	0%	
47	3-Methoxydiphenylamine	364.36	363.4	100a/o	0%	
48	2,2'-dipicolylamine	364.36	363.4	51 %	49%	
49	N-Methyl-2-(4-Nitrophenyl)ethylamine	345.2	344.2	88%	12%	
50	6,7-dimethoxy-1,2,3,4-terthydroiso	394.82	393.8	88%	12%	

Kinetic studies of macroscale nucleophilic aromatic substitution. Ten amines (#3, 13, 16, 19, 21, 23, 32, 33, 48 and 49) with various %conversions (from Table 1) were selected for time-course kinetic studies. Each of the amine solutions (0.1M, 0.102mmol) in 1-methyl-2-pyrrolidinone (1.02mL) was added to a 1.5mL-glass vial containing resin 2 (20mg, 0.034mmol). The reactions were allowed to shake at room temperature for 0.5, 1, 2, 4, 8 and 24 h. Ten beads were removed and transferred to a glass vial at each time point. After rinsing with DCM, MeOH and DCM, the resin beads were cleaved with 70% TFA/DCM (100μL) at room temperature for 3 h. The cleavage solutions were transferred and dried. The products were redissolved with methanol and analyzed by HPLC and LC-MS. The results are shown in Table 2 and Fig. 9.

Table 2. The results of kinetic studies of macroscale nucleophilic aromatic substitution

Reaction	Amines									
Time h	#3	#13	#16	#19	#21	#23	#32	#33	#48	#49
0.5	100	324	30.2	2	25.1	48.8	0	100	14.9	11
1	100	49.4	47.4	3.3	39.8	66	0	100	21.2	14.1
2	100	64.1	60.8	5.5	51.6	80.6	0	100	30.4	18
4	100	79	79.8	8.8	69.7	91.9	0	100	34.6	20.9
8	100	92.4	93	17.7	84.3	94.1	0	100	38.8	22.5
24	100	94.8	96.7	426	921	96.8	0	100	39.7	28

Kinetic studies of microscale nucleophilic aromatic substitution on Mark 20 v.1.0 Microfluidic Processor using Chemtel™ 144 chip. Two polystyrene beads were placed in each individual reaction well. A cassette consisting of a 144 chip, a gasket and a well plate was assembled on Mark-20 v1.0 Macrofluidic Processor, ready for parallel synthesis.

Each of 0.1 M secondary amine solutions in NMP (500 μL) was dispensed into a clean and dry

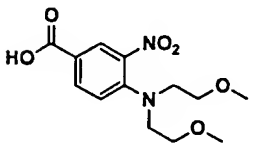
reagent vial, and then placed into the designated position on the row reservoir manifold. The order of ten secondary amines was designated as follows: N-methylpropylamine#3 (Row-2), 2-ethylpiperidine#13 (Row-3), 2-piperdinemethanol#16 (Row-4), N-allylcyclopentylamine#19 (Row-5), bis(2- methoxyethyl) amine#21 (Row-6), N-ethylbenzylamine#23 (Row-7), 3-(3-pyridylmethylamino) propionitrile#32 (Row-8), 1-(2-pyridyl) piperazine#33 (Row-9), 2,2'-dipicolylamine#48 (Row-10) and N-methyl-2-(4-(nitrophenyl)ethylamine#49 (Row-11). Low-pressure nitrogen source was applied to fill fluid through the row sequentially. After completion of line filling, high-pressure nitrogen source was applied to fill the reagents into the reaction wells in each row in a sequential fashion two through eleven. The nucleophilic aromatic substitution reaction was allowed to carry on for 0.5, 1, 2, 4, 8 and 24 h. The fluid was removed from all wells by employing vacuum. The chip and lines were continuously dried under vacuum.

The beads from each well were manually transferred to a glass vial and treated with 70% TFA/DCM for 3 h to affect cleavage. The cleavage solutions were concentrated and redissolved with methanol. The products were analyzed by HPLC and LC-MS. The results were shown in Table 3, Figs. 10, 11 and 12.

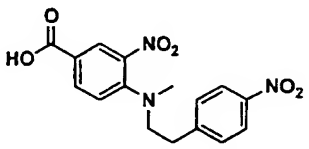
Table 3. The results of kinetic studies of microscale nucleophilic aromatic substitution

Reaction	Amine#21		Amine#48		Amine#49	
Time h	Microscale	Macroscale	Microscal	Macroscale	Mi os le	Macroscale
0.5	34.4	25.1	26.5	14.9	27.1	11.0
1		39.8	54.5	21.2	25.9	14.1
2	63	51.6	68.9	30.4	42.6	18.1
4	84.6	69.7	79.8	34.6	83.2	20.9
8	100	84.3	88.6	38.8	73.7	22.5
	*Microscale: 17 nmol; Macrosdes:		34 Nmol			

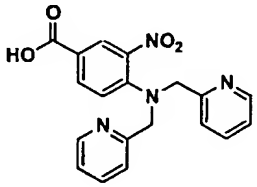
The structures of three products from Amine#21, 48 and 49 are shown below.



Product# 21



Product# 48



product# 49

These examples of nucleophilic aromatic substitution on a solid support clearly show improvements in yield, rate of reaction, and purity of material produced due to chemistry conducted at the micro level (less than 1 microliter). The known benefits of being at the microscale include better surface to volume ratio of reagents and reaction containers with these microfluidic chips, as well as improvements in mixing and heat exchange. For every 10 fold decrease in reaction volume a 100 fold improvement in mixing and heat exchange is encountered.

To those skilled in the art, it is obvious that other chemical processes would clearly benefit from moving from microscale to macroscale. In a general sense these include reactions that are extremely exothermic, reactions requiring large surface to

volume ratios such as heterogenous catalysis and rapid reactions such as DNA synthesis, where mixing may limit reaction time and efficiency (as a function of equivalents of reagent used). For heterogeneous catalysis a well plate of the catalyst, or glass/silicon or plastic coated with catalyst, (such as Pd, Pt, Ni or metal amalgams-raney Ni, CuNi) with a gas such as hydrogen, carbon monoxide or ammonia under pressure would be expected to benefit from these reduced volumes. It is expected that chemistry conducted without a solid support would also benefit from being in a microscale, since the presence or absence of a solid support is not a prerequisite for the enhancement.

While particular embodiments of the invention have been shown and described, numerous variations and alternate embodiments will occur to those skilled in the art. Accordingly, it is intended that the invention be limited only in terms of the appended claims.

What Is Claimed Is:

1. A method for performing a reaction in a microreactor having a plurality of input rows and columns coupled to reaction wells, said method comprising the steps of:

coupling said rows of wells with a first coupling agent;

coupling a second of M rows of wells with a second coupling agent;

forming similar chemical compound in parallel in each of the wells.

2. The method as recited in claim 1 further comprising stopping the flow of reagents in to the wells prior to the step of forming.

3. The method as recited in claim 1 wherein said well is coated with a catalytic material.

4. The method as recited in claim 1 wherein said catalytic material is composed of one selected from the group of palladium, platinum, copper-nickel amalgams and nickel.

5. The method as recited in claim 1 wherein said columns and rows of wells of said fluid processing device are filled by pressure pumping.

6. The method as recited in claim 1 wherein the fluid processing device is being used for peptide synthesis.

7. The method for addressing wells as recited in claim 1 wherein the fluid processing device is being used for hydrogenations.

8. The method for addressing wells as recited in claim 1 wherein the fluid processing device is being used for hydroformylations.

9. The method for addressing wells as recited in claim 1 wherein the fluid processing device is being used for DNA synthesis.

10. The method for addressing wells as recited in claim 1 wherein the fluid processing device is being used for an aromatic substitution.

11. The method for addressing wells as recited in claim 1 wherein the fluid processing device is being used with a solid-support bound core.

12. The method for addressing wells as recited in claim 1 wherein said first and second coupling agents are selected from the group comprising N-protected amino acids, diisopropyl carbodiimide, and peptide coupling agents.

13. The method for addressing wells as recited in claim 1 further comprising the step of placing beads into the wells.

14. The method for addressing wells as recited in claim 1 wherein at least said microreactor has 96 wells.

15. The method for addressing wells as recited in claim 1 wherein the volume of each well is less than about 3 microliter.

16. The method of addressing wells as recited in claim 1 wherein said first coupling agent comprises a solution.

17. The method of addressing wells as recited in claim 1 wherein said second coupling agent comprises a solution.

---

18. A method performing an aromatic substitution in a microfluidic device having a plurality of input rows and columns coupled to reaction wells comprising the steps of:

placing preloaded beads into wells;

coupling secondary amines to rows of wells;

coupling a second of M rows of wells with a second coupling agent;

forming similar a compound in parallel on the beads in each of the wells.

19. A method as recited in claim 18 further comprising the steps of cleaving the compound from the beads.

20. The method for addressing wells as recited in claim 18 wherein said columns and rows of wells of said fluid processing device are filled by pressure pumping.

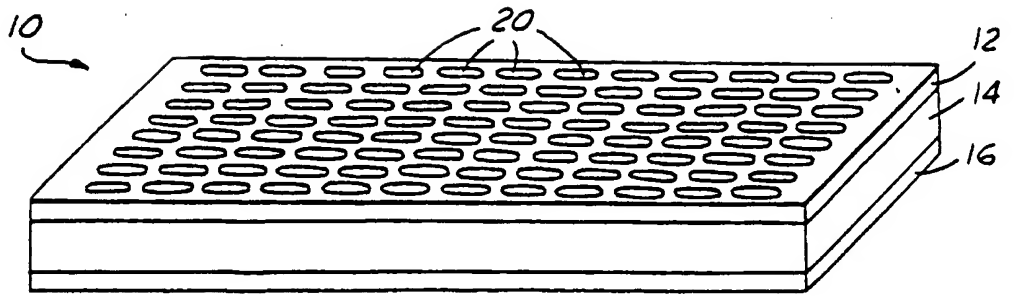


FIG. 1

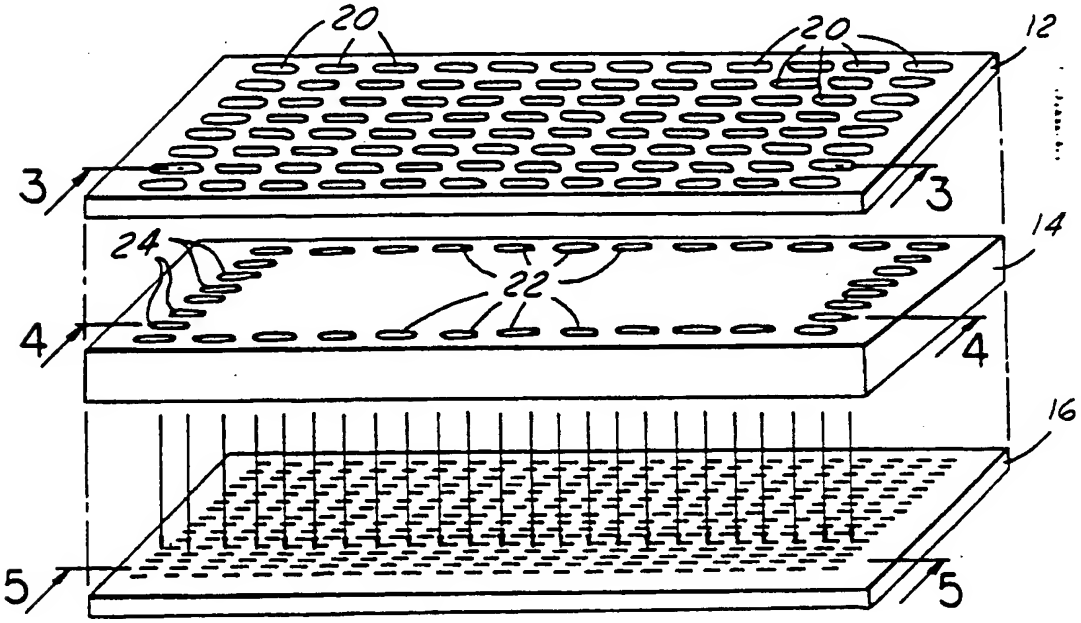


FIG. 2

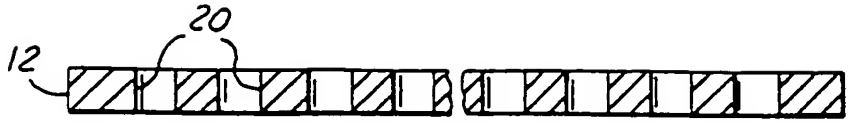


FIG.3

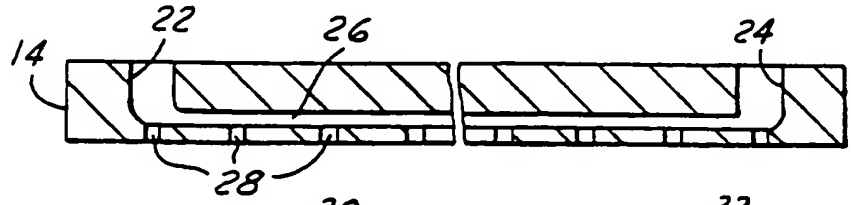


FIG.4

FIG.5

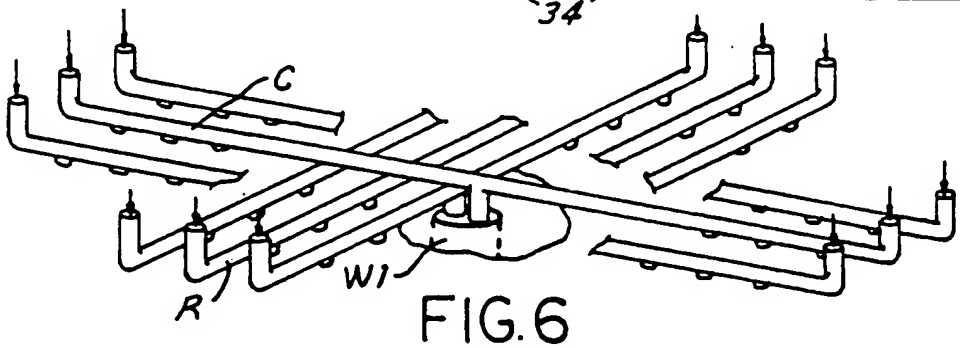


FIG.6

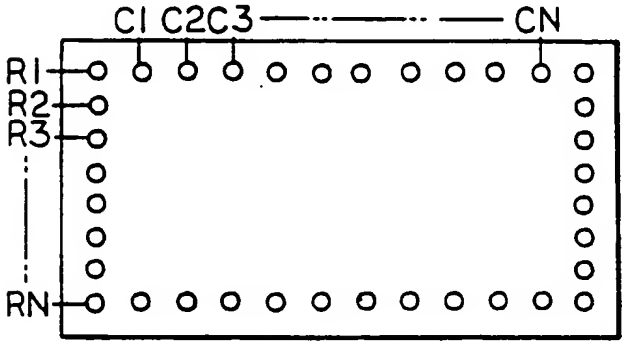


FIG.7

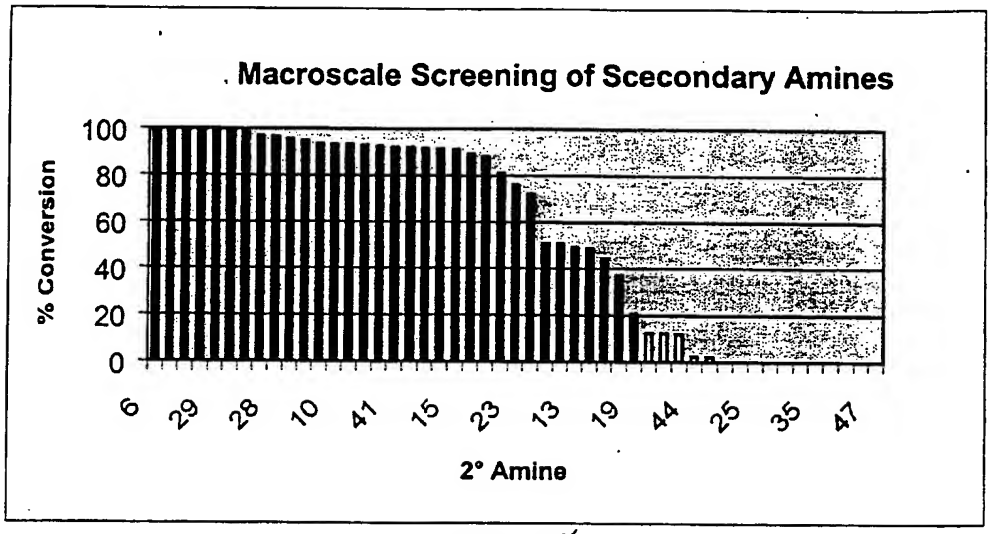

Macroscale Screening of Scecondary Amines
% Conversion
100
80
60
40
20
0
6
29
28
10
41
15
23
13
19
44
25
35
47
2° Amine

Fig 8

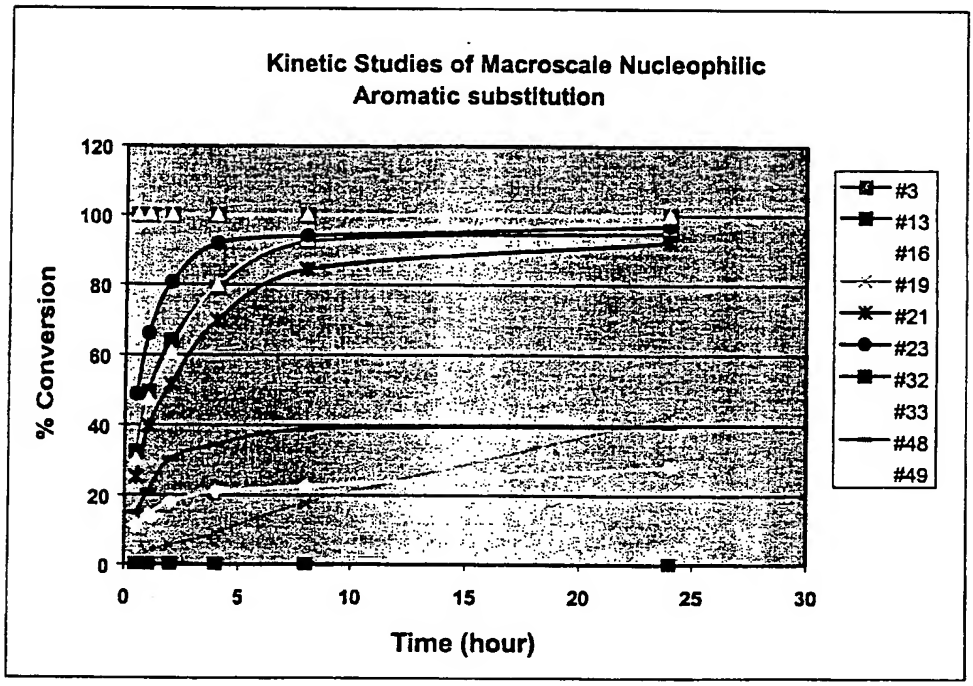

Kinetic Studies of Macroscale Nucleophilic Aromatic substitution
% Conversion
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (hour)
#3
#13
#16
#19
#21
#23
#32
#33
#48
#49

Fig 9

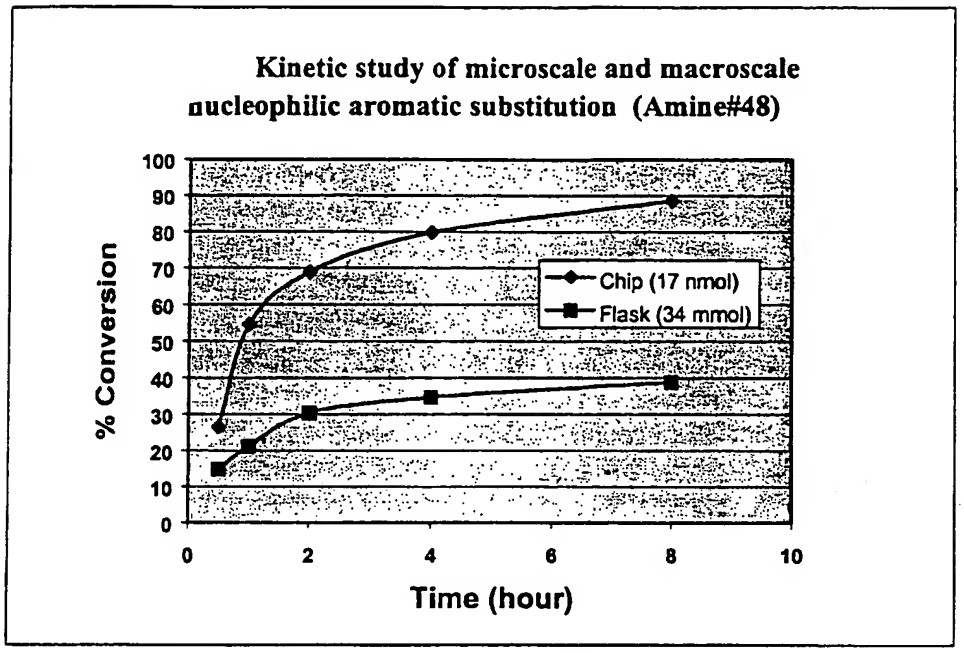

Kinetic study of microscale and macroscale nucleophilic aromatic substitution (Amine#48)
% Conversion
Time (hour)
Chip (17 nmol)
Flask (34 mmol)
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10

FIG 10

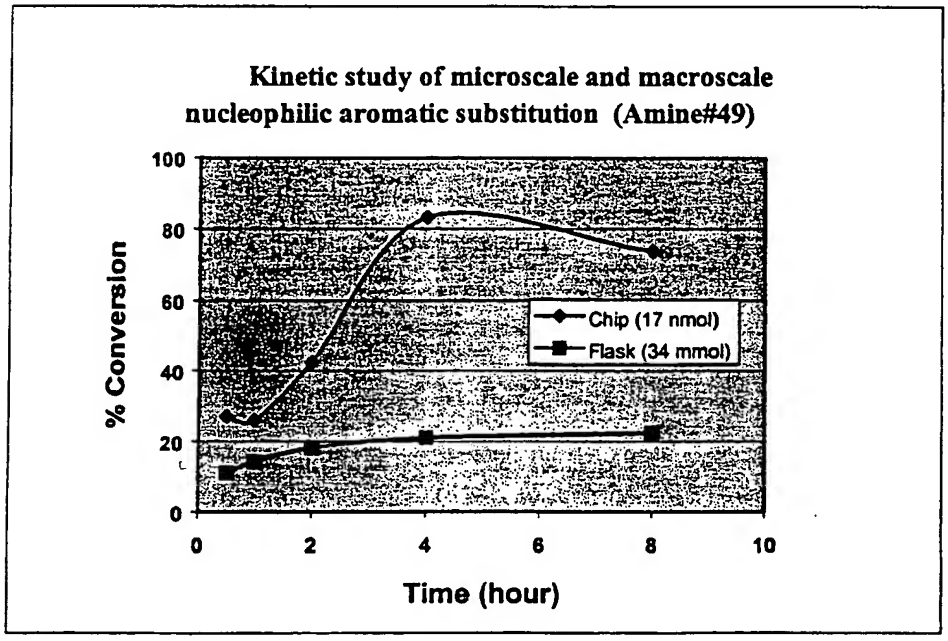

Kinetic study of microscale and macroscale nucleophilic aromatic substitution (Amine#49)
% Conversion
Time (hour)
Chip (17 nmol)
Flask (34 mmol)
0
20
40
60
80
100
0
2
4
6
8
10

FIG 11

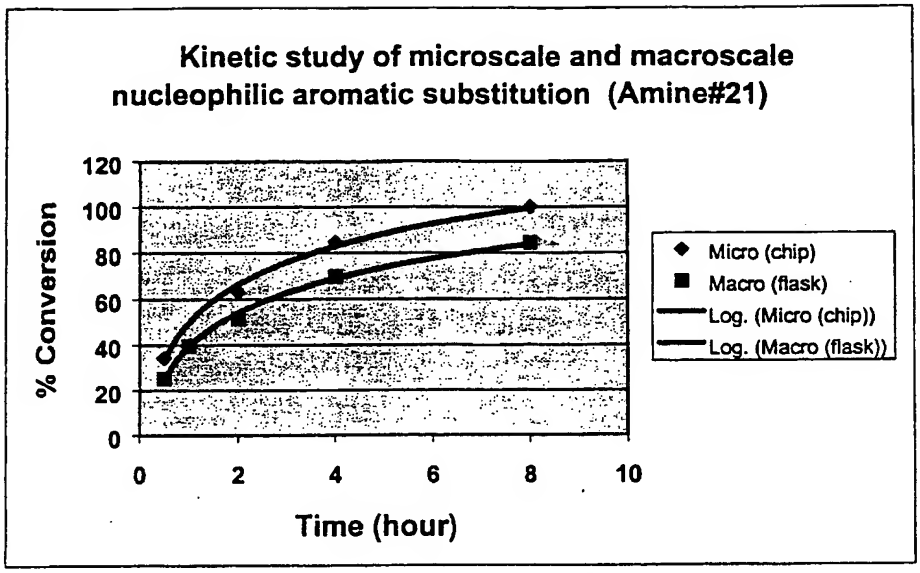

Kinetic study of microscale and macroscale nucleophilic aromatic substitution (Amine#21)
% Conversion
120
100
80
60
40
20
0
0
2
4
6
8
10
Time (hour)
Micro (chip)
Macro (flask)
Log. (Micro (chip))
Log. (Macro (flask))

FIG 12